a DNA segment encoding naturally occuring mammalian thrombopoietin, wherein the DNA segment [comprises] encodes a polypeptide comprising a region that is at least 80% identical to the region between [nucleotide number 237 and nucleotide number 722 of SEQ ID NO:1] amino acid number 45 and amino acid number 206 of SEQ ID NO:2, and wherein said thrombopoietin stimulates MPL-dependent cell proliferation; and

a transcription terminator.

B3 word.

In claim 24 at line 3, please delete "the" and insert therefor, --said--.

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33. (Amended) A method according to claim 32 wherein said cell is a eukaryotic cell and wherein said hematopoietic protein is secreted by said cell and is recovered from a medium in which said cell is cultured.

B4

REMARKS

Reconsideration of the application in view of the above amendments and following remarks is requested. Claims 10, 11, 13, 20, 22-24, 27, 28, 32, and 33 are now in this case. Claims 10, 20, 24, and 33 have been amended. No claim is allowed.

Applicants' representative thanks Examiner Spector for the courtesy extended in the interview of October 26, 1995. Claims 10, 20 and 33 have been amended as discussed in that interview.

The specification has been amended to address certain formal matters raised in the Office Action. No new matter has been added. For the Examiner's convenience, a replacement page 129, containing the new Abstract of the Disclosure, is included herewith.

The Examiner rejected claims 20, 22, 24, 28, 32, and 33 under 35 U.S.C. § 112, first paragraph, in the belief that the disclosure is enabling only for claims limited in scope to expression vectors and processes for

the expression of thrombopoietin in eukaryotic cells. The Examiner referred to page 18 of Applicants' specification, which indicated that the carbohydrate associated with the second domain of TPO is involved in proper assembly and secretion of the protein.

Applicants respectfully traverse this grounds of rejection. The passage cited by the Examiner states that the carbohydrate associated with the second domain is involved in, not required for, proper assembly and secretion. As disclosed in Example XIII of the specification, the cytokine domain alone (i.e., a truncated TPO polypeptide lacking the carbohydrate-rich second domain) can be expressed in and secreted from *S. cerevisiae*, thereby demonstrating that the carbohydrate-rich second domain is not required for proper assembly and secretion of TPO. The level of secretion of the cytokine domain alone is relatively low, suggesting that the second domain plays a role in promoting assembly and secretion.

Assembly and secretion are not of concern when expressing TPO in prokaryotic hosts. As disclosed on page 26, heterologous proteins expressed in bacteria are commonly retained in the cytoplasm as insoluble granules, which are recovered and denatured, and the protein is re-folded *in vitro*. As further evidence of the enablement provided by Applicants' disclosure, the Examiner's attention is directed to WIPO publication WO 95/18858 and European Patent Office publication 668,352, both of which were included with Applicants' Supplemental Information Disclosure Statement filed November 1, 1995. WO 95/18858 discloses methods for producing TPO in prokaryotic hosts on pages 57-60 and in Examples 22 and 23. Expression of TPO in prokaryotic hosts is also disclosed in EP 668,352, for example at pages 19-20, 21, and 96-97. These methods are generally disclosed by Applicants in their specification on page 26, wherein the protein is retained in the cytoplasm of the host cell in insoluble granules, which are recovered and subjected to protein denaturing and refolding.

Claim 33, which is directed to a method of secreting TPO from a host cell, has been amended to recite that the cell is a eukaryotic cell.

Claims 10, 11, 13, 20, 22-24, 27, 28, 32, and 33 were rejected under 35 U.S.C. § 112, second paragraph, as being indefinite in the recitation of "80% identical".

For purposes of clarity, claims 10 and 20 have been amended to recite that the polynucleotide encodes a polypeptide comprising a region that is at least 80% identical to the region between amino acid number 45 and amino acid number 206 of SEQ ID NO:2. The remaining claims include this limitation through their dependence on, or recitation of, claim 10 or claim 20. The recited amino acids are encoded by the polynucleotide coding region previously recited in these claims. The level of amino acid identity between two sequences is determined by conventional methods as disclosed on pages 14-15 of Applicants' specification. Briefly, sequences are aligned using any of several well known algorithms that apply the "blosum 62" scoring matrix to find the optimum alignment score. Once the optimum alignment is determined, the percent identity of the sequences is determined by the formula presented at the bottom of page 14. Because the optimum alignment score is used and the denominator of the ratio is the length of the longer sequence plus gaps, the ambiguity suggested in the alignments presented at the bottom of page 3 of the Office Action is not present. In the example presented by the Examiner, the identity is 4/6, or 67%, because the optimum alignment is 4, and the longer sequence is 6.

The Examiner believed that claim 23 was indefinite in the recitation of a secretory signal sequence operably linked to the DNA segment. Applicants respectfully disagree; the use of the term "secretory signal sequence" in claim 23 is consistent with the specification at page 23 and elsewhere. "Secretory signal sequence" is used in the specification to denote a DNA

segment that encodes a secretory signal peptide. Applicants respectfully submit that claim 23 as pending is definite and clear when read in light of the specification, and that amendment of claim 23 could create confusion.

Claim 24 has been amended to recite "said DNA segment" as suggested by the Examiner.

The references made of record with the Office Action have been reviewed. It is believed that the claimed invention is patentable over all art of record.

On the basis of the above amendments and remarks, Applicants believe that each rejection has been addressed and overcome. Reconsideration of the application and its allowance are requested. If for any reason the Examiner feels that a telephone conference would expedite prosecution of the application, the Examiner is invited to telephone the undersigned at (206) 442-6673.

Respectfully Submitted,

Gary E. Parker
Registration No. 31,648

Enclosures:
Amendment Fee Transmittal (in duplicate)
Postcard
Replacement Page 129